Attachment

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. Registration number: 14-R-0192

2. Number of animals used under Column E conditions in this study. 55

3. Species (common name) of animals used in this study: Guinea pig

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (Cut/paste from the approved docket and or amendments).

Mice and guinea pigs are the two major animal infection models established for the human herpes virus-2 (HSV-2) to evaluate protective efficacy of potential HSV-2 vaccines. Previously, we have successfully obtained satisfactory immune protection results using Balb/c mice that were immunized with our novel immunization strategy and challenged with HSV-2 viruses afterwards. To further prove the potency of the immunization regimen in another established animal model, the Hartley guinea pig was chosen to observe clinical signs, body weight and mortality post immunization and HSV-2 infection. The guinea pig is also a good model to study immune protection efficacy against recurrence of HSV-2 infection.[1] Females were infected with HSV-2 virus by intra-vaginal inoculation followed by treatment with test vaccines by intramuscular and intranasal routes of administration. Post infection, animals were monitored daily for clinical signs of disease. The viral challenge of guinea pigs with HSV-2 can have varying clinical readouts from no clinical signs to morbidity. To evaluate the viral disease state we followed the established published literature for viral clinical scoring listed in the table below. Animals that reached a moribund state or exhibit a score of (4) or a loss of 30% (Amendment 5) body weight were immediately euthanized.

Observation	Score
No clinical signs of disease	0
Vaginal erythema	1
Single to a few modest herpetic lesions	2
Large or fused vesicles	3
Severe vaginal ulceration and paralysis	4
Found dead	5

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Analgesics may interfere with immune responses and physiological processes to the infection and the display of disease symptoms and make the interpretation of the raised immune protection results technically difficult. The measurement of clinical scores is vital to the determination of the efficacy of the vaccine. Therefore, we propose not to use analgesics that could interfere with

test results. For example, morphine is known to suppress the innate immune system and reduce severity of HSV-1 infection; the opioid derivative buprenorphine may have similar effects; the cyclooxegenase-2 (Cox-2) pathway is required for efficient herpes virus replication: NSAIDS down-regulate this pathway and Cox-2 inhibitors limit the replication of herpes viruses; Lidocaine destabilizes the HSV virion. Therefore, animals exhibiting pain or unrelieved distress as described above will be euthanized.

6. What if any federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113. 102):

Not applicable.